Final Scientific Report

Cover Page

BARD Project Number: IS-4072-07

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Project Title: o saponins present in model systems and legume bread modulate cholesterol absorption in vitro and in vivo?

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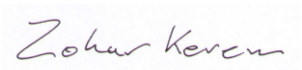
Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Abbreviations commonly used in the report, in alphabetical order**:**

Budget: IS: $144,000 US: $216,000 Total: $360,000



Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged				
Submitted, in review, in preparation	1		2	3
Invited review papers				
Book chapters				
Books				
Master theses			1	1
Ph.D. theses		1	1	2
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	2	1		3
Longer Visits (Sabbaticals)				

Description Cooperation:

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted	1			1
Issued (allowed)				
Licensed				

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RESEARCH OBJECTIVES

The overall general goal of the research project was to provide evidence for the beneficial biological activity of saponins from edible legumes, and to incorporate them into bread hat will become a functional food. Its functionality is in its ability to attenuate the absorption of dietary lipids and cholesterol.

RESULTS

Several conclusions and achievements can be demonstrated by the completion of the project:

1) Methods to efficiently extract, isolate and concentrate saponins from chickpea and soy were developed, that allowed the isolation and detection of at least 17 saponins in soy ingredients (A, B, E and DDMP type), and only Bb and βg in the chickpea counterparts. The latter is an additional support to preliminary results, and makes chickpea an attractive molecule for in depth basic research on the modes of action. The methods of isolation are a part of pending patents, and a decision needs to be made as to whether they serve or harm the patentability.

2) Accurate analytical techniques were developed for identification and quantification of soy and chickpea saponins in food ingredients and biological samples by HPLC-PDA and LC-MS. These are accompanied by a still ongoing research to determine the best protocol for rapid analysis using NIR spectroscopy that will allow on-line quality control, if and when products become commercial.

3) Effect of saponins on lipid absorption was assayed using mice as a model. The saponins concentrates were used as a nutritional additive in a mice feeding experiment, where we tested their effect on weight gain and lipid absorpption. We also examined the absorption of the saponins themselves in the GI tract. In a three weeks experiment, 80 mice consumed chow or high fat diets, with or without the addition of 3% (w/w) saponins from either soybean, chickpea or fenugreek seeds. Weight and food consumption were recorded, samples of urine and feces were collected during the experiment, and blood and tissue samples were collected on the last day of the trial.

The addition of saponins to the diet did not cause a change in total food consumption, as compared with the control group, but did induce weight loss in mice that were fed high fat diet. In mice fed normal chow diet, there was no change in weight in the groups treated with soybean or chickpea saponins, however, weight gain was markedly reduced in mice treated with fenugreek saponins. Saponins from all three legumes reduced total plasma cholesterol and triglycerides when consumed with high fat diet.

Analyses of saponins in the jejunum, and in collected urine and plasma revealed, for the first time that fenugreek saponins, unlike the other saponins studied here, are absorbed in the GI tract. Using MS in the MRM mode showed 7 knownsaponins and 1 known aglycone in all the above samlpes. Moreover, several molecules that are likely to be saponins, based on their mass spectra, were also detected in these sapmles. Analysis of feces showed that saponins are also secreted intact in both chow and high-fat diets. This may suggest that the doses used here are higher than required for the biological activity.

We assume that the mechanisms of their action are diverse and may include an interaction with lipids from the diet or with the bile salts that are secreted as a response to the high fat diet. This is the first report on the specific biological activity of saponins from chickpea and fenugreek.

4) In order to further identify the nutraceutical and pharmacological potential in these saponins, follow up experiments were performed to determine the possible mechanisms of action. One such mechanism, which was proposed in our grant application is micellarization of cholesterol. However, no significant effect of saponins on cholesterol micellarization was observed during in vitro digestion, thus suggesting an alternate path for their proposed hypocholesterolemic activity. Some loss occurred upon in vitro digestion: recovery in digesta was 60% for type A, 100% for type B, 100% for type E and 90% for DDMP type suggesting minimal degradation and partial conversion of the DDMP type during digestion. Higher recoveries were observed for soy bread as compared to chickpea containing breads, thus suggesting a matrix effect on saponin stability and bioaccessibility. Micellarization rate was higher for DDMP type (80 vs. 60%) and low uptake of saponins by Caco-2 cells was observed with predominance of B type (1-3% test medium).

5) Bread preparation was the final goal of this project. We aimed at preparing a palatable and health beneficial bread, amended with fractions of chickpea and soy powder. Fractions that were prepared are concentrated saponins from each powder, as well as protein isolates. LC-MS analysis confirmed stability of saponins during bread making with the exception of type E saponins (~30% recovery). Saponin addition affected texture of baked goods differently, depending on the product and the system chosen. Soy blend addition to pocket-type flat doughs resulted in soft, yet tough and rubbery texture. Increased “freezable” water (from 7.0 to 16 g water/100 g sample) was observed thus depicting poor plasticization of the gluten-starch network. When soy saponins were added as an extract to soy bread, a dramatic reduction of hardness during 7 days ambient storage was observed, thus suggesting anti-staling properties of these compounds. Chickpea saponins (1% addition) in the form of solvent extract resulted in harder texture of the wheat breads, significant loaf volume increase and lower “freezable” water content. The isolate contained also water soluble fiber that may have contributed to such changes. Reformulation of soy bread with chickpea protein isolate resulted in harder and denser breads at 2/3 substitution.
Modification of th bread to better resemble the original formulation resulted in higher quality breads. The formulation of these breads is evaluated for patening at the present time.